Phally polymorphism and reproductive biology in *Ariolimax (Ariolimax) buttoni* (Pilsbry and Vanatta, 1896) (Stylommatophora: Arionidae)*

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Abstract: Phally polymorphism, whereby some individuals in a hermaphroditic species have a complete functional penis (euphally), whereas others lack a penis (aphally) or have a reduced, non-functional penis (hemiphally), has evolved many times in pulmonate gastropods. Since the discovery of apophallation (penis amputation) in *Ariolimax* Mörch, 1860, aphallates in this genus have been attributed to apophallation. In laboratory studies in *Ariolimax (Ariolimax) buttoni* (Pilsbry and Vanatta, 1896), we found aphally in juveniles as well as in individuals reared in isolation to adulthood, demonstrating that the aphallate condition is not always due to apophallation. Some aphallate individuals reared in isolation from hatching produced eggs and viable hatchlings, providing the first demonstration of uniparental reproduction in this species. Egg-to-egg generation time in laboratory-reared individuals ranged from 10 months to more than 24 months. Anatomical data also elucidate the reproductive cycle of this species. Four reproductive states have been identified by the appearance of the reproductive system. Spring and early summer populations consist of individuals in the immature and intermediate reproductive states. The hypertrophied state was found from autumn until early spring. Egg-laying occurred in the laboratory in the fall and winter. Copulation consists of unilateral or simultaneously reciprocal intromissions and occurred in the laboratory between February and September. Very long copulations (more than 7 h) are more frequent than in other species of *Ariolimax*. Phally polymorphism, uniparental reproduction, and the variation in generation time should play important roles in determining the variance of mating success and the potential for sexual selection in this hermaphroditic species.

Key words: gastropod, genitalia, aphally, life history, self-fertilization

Banana slugs, giant stylommatophoran slugs of the genus *Ariolimax* Mörch, 1860, are common and conspicuous members of temperate rain forests and other mesic habitats of the northwestern coast of North America. Although banana slugs are well-known and popular with the general public, remarkably little is known about their biology (but see Harper 1988, Leonard *et al.* 2002, Cody 2006, Pearson *et al.* 2006). In *Ariolimax,* as in stylommatophorans in general, taxonomy has been based on genital characters (Pilsbry 1948). Eberhard (1985) suggested that where genital characters have evolved rapidly enough to distinguish species (and even subspecies), sexual selection has played an important role in the evolution of the group. The genus *Ariolimax* offers a particularly good opportunity to test this hypothesis since it consists of a small number of taxa (Roth and Sadeghian 2003, Pearse *et al.* 2007), most of which are found in coastal Central California in quite similar habitats, but which have very divergent genitalia and sexual behavior (Leonard *et al.* 2002, 2005). Accurate information about the reproductive biology of *Ariolimax* spp. is necessary to perform such a test.

One of the most mysterious aspects of *Ariolimax* biology is the existence of aphallate individuals and the extent to which these can be explained by apophallation. Apophallation is a behavior first observed in *Ariolimax (Meadarion) californicus* (Cooper, 1872) (Heath 1916, Leonard *et al.* 2002) and subsequently in *Ariolimax (Meadarion) dolichophallus* Mead, 1943 (Mead 1942, 1943, Harper 1988, Leonard *et al.* 2002) whereby the penis is sometimes chewed off at the end of copulation in these simultaneous hermaphrodites. Since Heath (1916) most authors have been content to explain aphallate individuals of *Ariolimax* as the product of apophallation. However, Heath himself expressed doubt, saying, "years ago I visited Hog Island in Tomales Bay, and found over 400 specimens ... every one of the specimens was totally lacking a penis or any sign of one." (quoted in Mead, 1943, p. 685). Mead (1943) later collected in the same area, finding no slugs at Hog Island and an entirely phallate population nearby. Paull (1951) examined the gross anatomy of *Ariolimax buttoni* (Pilsbry and Vanatta, 1896) (= *A. columbianus* (Gould in Binny, 1951), see below) at Mills College in Oakland, California and found that of the 27 adult speci-

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mens she dissected, none had a penis. More recently, Roth (2004) reviewed anatomical descriptions of *Ariolimax* in detail, and suggested that the descriptions are inconsistent with what would be expected if a penis were amputated during copulation. He argued that apophallation should sever both the penis and vas deferens, leaving unconnected remnants of both and an intact penis retractor muscle, whereas Pilsbry and Vanatta's (1896) drawing of *Aphallarion buttoni* showed an intact vas deferens connected to the genital atrium by a short bulb with no penis and no penis retractor muscle. Roth (2004) concluded that the descriptions were more consistent with a phally polymorphism. Such phally polymorphisms are well-known and widespread in pulmonates, including stylommatophorans (Tompa 1984, Pokryszko 1987, Baur and Chen 1993, Doums and Jarne 1996, Viard *et al.* 1997, Doums *et al.* 1998, Gómez 2001), and there are even instances of a phally polymorphism having lead to an erroneous splitting of a single species into two genera (*e.g.*, Lace 1992).

Here, we present previously unpublished observations on aphallate and euphallate individuals in two populations of *Ariolimax (Ariolimax) buttoni* and data showing aphally in individuals which cannot have been involved in apophallation because they were reared in isolation to adulthood. These observations demonstrate that this species has a phally polymorphism, with both aphallate and euphallate individuals in some populations. We also present laboratory observations on the sexual behavior and life cycle of *A. buttoni* and document uniparental reproduction in this species.

MATERIALS AND METHODS

Taxonomy

The animals used in this study are *Ariolimax (Ariolimax) buttoni*. This species was first described by Pilsbry and Vanatta (1896) from a large series of aphallic ariolimacines from Oakland, California as a new genus. Subsequently, this taxon was synonymized to *Ariolimax (Ariolimax) columbianus* (Gould in Binny, 1951) (Waste 1940, Mead 1943, Pilsbry 1948) in the expectation that the aphally could be attributed to apophallation during copulation. This species was considered to be the only ariolimacine to include maculate individuals and was stated to have a range extending from Tuolomne County, Monterey County, the eastern shore of San Francisco Bay, and the city of San Francisco in California, north to southeastern Alaska (Pilsbry 1948, Roth and Sadeghian 2003). Recent molecular evidence suggests that *A. columbianus*, as defined by Mead (1943) and Pilsbry (1948), is not monophyletic but rather that populations of *Ariolimax* north of Mendocino County, California (the true *A. columbianus*, since the species was described from specimens collected near the Columbia River, see discussion in Pilsbry 1948) are evolutionarily distinct from the more southern populations (Leonard *et al.* 2005, Pearse *et al.* 2005). The name *Ariolimax buttoni* (Pilsbry & Vanatta, 1896) has been revived to designate the southern clades formerly included in *A. columbianus* (Pearse *et al.* 2007). *Ariolimax buttoni* and *A. columbianus* do not even represent sister clades (Pearse *et al.* 2007). Like *A. columbianus*, *A. buttoni* may be either maculate or immaculate. *Ariolimax (Meadarion) brachyphallus* Mead, 1943 is sympatric with *A. buttoni* in San Francisco but all individuals used in this study from San Francisco have been identified by molecular markers as belonging to *A. buttoni*. There are no reports of sympatric ariolimacines in Alameda, Sacramento, Mendocino, or Marin counties.

Animals

Mills College animals

Specimens were collected from March 1951 to February 1952 in two locations; the Mills College campus (37° 46' 41"N, 122° 10' 49"W) and the nearby Leona Heights Park (37° 47' 31"N, 122° 10' 41"W) in Oakland, Alameda County, California. Approximately 24 animals from each location were housed in the laboratory in terraria filled with damp earth and fed with lettuce. Other animals were dissected shortly after collection.

UCSC animals

Specimens were collected from five locations in Central California: (i) a wooded area of Mount Parnassus on the campus of the University of California, San Francisco, San Francisco County (37°45' 38"N; 122° 27' 28"W); (ii) a levee of Staten Island in the Cosumnes River, Sacramento County (38° 15' 56"N, 121° 26' 31"W); (iii) near Comptche, Mendocino County (39° 15' 54" N, 122° 35' 24" W); and two locations in Marin County, namely (iv) near Muir Woods in central Marin County (37° 53' 07"N, 122° 32' 26"W) and (v) the west side of Tomales Bay (38° 10' 25"N, 122° 55' 26"W) in western Marin County. Animals were maintained in the laboratory as groups or individuals in plastic boxes as described elsewhere (Leonard *et al.* 2002).

Anatomical studies

Mills College animals

A total of 67 individuals of *Ariolimax buttoni*, ranging from 16 to 55 grams in weight, were dissected. Three slugs were dissected at the beginning and middle of each month from March to May 1951. Five slugs were dissected every two weeks from October 1951 to February 1952. Two of the slugs were taken from the animals kept in terraria whereas the other three were collected from the field. The day before dissection, the slugs were weighed and the next day they were drowned, dried to remove excess mucus, pinned to a

dissecting tray and dissected in water. A longitudinal incision was made on the right side from the caudal pore to the mouth, the dorsal skin retracted, and the genitalia freed by severing connective tissue and the protractor muscles of the tentacles. After observation of the intact condition, the skin was cut around the atrium and the reproductive system was laid out separately for measurement. The data recorded were: a) the color and degree of development of the reproductive system; b) the position of the vas deferens and the presence or absence of a penis and/or terminal bulb on the vas deferens; c) the approx. width of the vagina and oviduct; d) the approx. length and width of the spermatheca; e) the approx. length and width of the ovotestis; and f) the approx. length of the albumen gland.

UCSC Animals

Anatomical studies were conducted with *Ariolimax buttoni* derived from two populations (Staten Island and Tomales Bay) and reared as described by Leonard *et al.* (2002). Three of the individuals were collected from the field and then maintained in the laboratory until their deaths, at which time they were frozen. The other individuals were hatched in the laboratory and frozen when slightly over 30 months of age. They were thawed just before dissection, pinned to a dissecting tray, and dissected under water. An incision was made through the body wall with scissors along the left side, just above the foot, and the dorsal body wall peeled back to expose the digestive system and genital organs, and the albumen gland, ovotestes, and male and female parts of the genital organs teased apart. The condition of the spermatotheca and the presence or absence of the penis was noted. If absent, the presence or absence of a penis stub was noted as well as the course of the vas deferens. The male and female portions of the genital organs were sketched for most specimens; in a few cases they were measured and photographed through the dissecting microscope, using an Olympus Camedia C-3040 digital camera. Upon completion, the dissected animals were preserved and archived in 10% formalin.

Rearing studies

Two sets of rearing studies have been conducted with *Ariolimax buttoni*. The first series was conducted at Mills College (Mills College animals) from 1951 to 1952 (Westfall 1960). Eggs and juveniles were maintained in terraria filled with damp earth along with the parents. They were fed lettuce leaves. In total 17 juveniles ranging in age from 1 day to 19 weeks were taken for anatomical study.

The second rearing study was conducted at the Long Marine Laboratory of the University of California-Santa Cruz (UCSC animals) from 2003 to 2006. Slugs were collected from Staten Island and Tomales Bay and held in group boxes. Eggs laid in fall and early winter 2003-2004 were maintained as described previously (Leonard *et al.* 2002) and hatchlings weighed when found and transferred to individual plastic containers. All containers were cleaned and lettuce added weekly. After the juveniles reached 5 g in weight, dry cat food was also given. Slugs were transferred to larger containers as necessary as they grew. They were weighed weekly until 6 mo and biweekly thereafter.

All hatchlings were reared in isolation until June 2004 when, as part of a mating study (Leonard, *et al.* unpubl. obs.), 40 individuals from the Staten Island group and 40 from the Tomales Bay group were randomly assigned to a mating treatment. Eight individuals, each from Staten Island and Tomales Bay parents, were assigned to the single animal (continued isolation) treatment. Equal numbers of individuals from Tomales Bay and Staten Island parents were also assigned to each of four other rearing conditions: a) paired with an individual from the same source population; b) paired with an individual from the other source population; c) in a group box with three others from the same source population; or d) in a group box with one individual from the same source population and two from the other source population. All of the individuals derived from the Staten Island population were spotted whereas all of the Tomales Bay descendants were immaculate. Redwood bark mulch was added to each box to encourage egg-laying. Slugs were weighed biweekly and cleaned and fed weekly (as in Leonard *et al.* 2002), so that all boxes were checked for eggs at least weekly. Clutches found were treated as described above. Hatchlings were weighed when found and preserved in 95% ethanol for future genetic analysis.

Behavioral observations

All observations on sexual behavior reported here for *Ariolimax buttoni* (see Table 3) resulted from casual observations of UCSC animals held in group boxes. Where animals were observed to be copulating when the box was opened for cleaning, notes were made and/or the behavior was videotaped as time allowed. Where possible, observations were continued for 30 min after the end of the copulation. The copulation of Mendocino individuals on April 10, 2001 was noted but not followed for any length of time.

RESULTS

Phally status

Mills College animals

Only 7 of the 67 Mills College slugs (4 of the 55 slugs collected on the Mills College campus, and 3 of the 12 slugs collected at Leona Park), were found to have a penis and, of

those, one only had a penis fragment (Table 1). In 59 of the 60 individuals lacking a penis, the vas deferens ended abruptly in the atrial mesentery (Fig. 1). In 20 of these 59 individuals, there was a small bulb at the end of the vas deferens as described by Pilsbry and Vanatta (1896). The apical termination of the vas deferens in the individual with a fragment of a penis was on the penis fragment. This specimen was in the immature reproductive state (see below). None of the 17 Mills College juveniles hatched in the laboratory had a penis.

UCSC animals

A total of 16 individuals were dissected (Table 2). All of the individuals derived from Tomales Bay populations and the individuals collected from UCSF and Central Marin had a fully developed penis with an apical retractor muscle and a long vas deferens that terminated in the penis (Fig. 2B). None of the 10 individuals derived from the Staten Island population had a penis (Fig. 2A), including two individuals that spent their entire lives in isolation. Of these 10 individuals, 5 had a small stub where the penis would be in a euphallate individual (Fig. 2A) whereas the remaining 5 lacked any trace of a penis (Fig. 1). All 10 individuals lacked a penial retractor muscle and 9/10 had a blind termination of the vas deferens. In one individual the vas deferens ended in a bulb as was seen in many of the Mills College animals (Table 1). The vas deferens connected to the base of the penial stub in one individual.

Reproductive development

Mills College animals

Measurements and appearance of the individual organs of the reproductive tract for the 67 dissected adult Mills College slugs are given in Westfall (1952). On the basis of the color and appearance of the organs and the length of the albumen gland, four reproductive states were identified: 1)

immature, 2) intermediate (or "sperm producing"), 3) hypertrophied (or "egg-laying"), and 4) "old" (Appendix). The reproductive state in the dissected slugs varied with season (Fig. 3). In the spring most (10/12) individuals were in the intermediate state (Fig. 3) with an albumen gland of small to intermediate size. In contrast, in the fall and winter there was a clear division into two groups of individuals: those in the immature state with a very small albumen gland and those in the hypertrophied state with a very large albumen gland. Slugs collected in October and kept in captivity were also found to be more often in the hypertrophied state as the date of dissection moved from November to February.

There was no relationship between season or reproductive state and the presence of a penis. Four of the individuals with a penis were in the immature reproductive state, one in the intermediate state, and two others in the hypertrophied state (Appendix; Fig. 3). In addition, there was no relationship between body weight and the presence of a penis; the second smallest and third largest animals dissected had a penis (Fig. 4). Each reproductive state included individuals with a broad range of weights although the smallest animals were in the immature state and the largest in the hypertrophied state (Fig. 4).

The reproductive tract was not distinguishable in juveniles for two weeks after hatching. After two weeks, the organs of the reproductive system were in the immature state with a hair-like hermaphroditic duct, leading from a small, white, smooth, lobed ovotestis and a small albumen gland. After a short distance this duct divided into a free oviduct and a vas deferens. These ducts were straight and hair-like in younger specimens but began to show more coiling in older specimens. In older juveniles the vagina and vas deferens terminated jointly in the atrium (as in Fig. 1) but there was no indication of a penis even at 19 weeks of age when other reproductive organs were well developed. Juvenile slugs of 15-19 weeks of age weighed between 3 and

Table 1. Summary of morphological data on adult *Ariolimax buttoni* from Mills College in Oakland, California

Phally status	Number of slugs	Weight (mean ± SD)	Number in immature state*	Number in intermediate state*	Number in hypertrophied state*	Number with state unclear
Penis present	6	32.12 ± 129 g	3	0	2	1 (state questionable)
Penis fragment	1	37.5 g	1	0	0	
No penis but bulb on end of vas deferens	20	32.4 ± 9.08 g	2	9	8	1 (state questionable)
No penis and no bulb	39	29.3 ± 5.62 g	18	13	8	
Unknown	1	24.5 g				(insufficient information)
Weight (mean ± SD)			28.42 ± 6.61 g	30.49 ± 6.27 g	33.31 ± 9.52 g	32.53 ± 9.91 g
Total	67		24	22	18	3

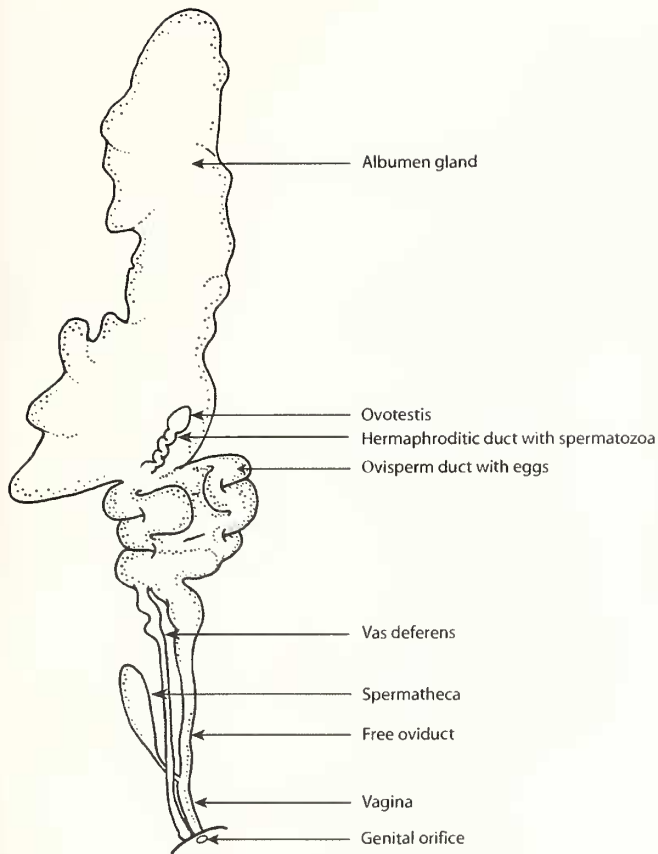* See Appendix for definition of reproductive states.

Figure 1. Drawing of a dissected aphallate individual of *Ariolimax buttoni* which had been observed to lay eggs before dissection; hypertrophied reproductive state (see Appendix) with sperm present in the hermaphroditic duct and the oviduct distended with eggs. The hermaphroditic duct proceeds from the ovotestis (=hermaphroditic gland = gonad) carrying both eggs and sperm. After the albumen gland, the term ovisperm duct is used for a convoluted conjoined vas deferens and oviduct. After the vas deferens branches off (to the penis in euphallate specimens, see Figure 2B) to end near the atrium here, the ovisperm duct continues as the free oviduct to the point of attachment of the spermatheca (=bursa copulatrix = gametolytic gland) with the vagina. The free oviduct is usually a wide, convoluted duct while the vagina is a short, straight tube from the spermatheca to the atrium, and is divided into two portions by a small, thick, annular muscle (intrinsic muscle of the vagina).

6.3 g (Westfall 1960). The juvenile slugs all showed the immature reproductive state (Appendix), except that the very first juvenile examined, dissected at four weeks of age (representative weight, 0.7 g, Westfall 1960), was found to have a relatively large, loosely lobed, ovotestis. The reason for the different appearance of this one individual is not known.

UCSC animals

The spermatheca was reddish in most individuals from Staten Island. All Tomales Bay animals had a small spermatheca and in 3 of the 4, it was pale in color. While all of the isolated, dissected animals from Staten Island (3/8), laid one or more clutches of eggs, none of the isolated Tomales Bay animals laid eggs (see below).

Egg-laying

Results on egg-laying and hatching in the Mills College animals have been published elsewhere (Westfall 1960). In UCSC animals, egg-laying by animals hatched in the fall-winter of 2003-2004 from Staten Island and Tomales Bay parents, began in October 2004 and continued until late February 2005. Egg-laying then paused for this group of slugs until mid-August 2005 when it resumed and continued until late February 2006. However, only in one box of four individuals did egg production occur in both seasons (2004-2005 and 2005-2006).

Three individuals from Staten Island parents, isolated since hatching, laid eggs (only two were dissected and listed in Table 2). One individual, from a clutch of 13 November 2003, produced three clutches for a total of 89 eggs: 32 eggs on 13 December 2004 (Clutch 1A), 13 eggs on 20 December 2004 (Clutch 1B), and 45 eggs on 9 February 2005 (Clutch 1C). This individual was found dead on 4 August 2005. All three clutches developed normally and produced a total of 45 viable hatchlings. Clutch 1A produced 21 hatchlings between 31 January and 7 February 2005. Clutch 1B produced 6 hatchlings from three eggs between 3 and 9 February 2005: one egg produced a single hatchling, one produced twins, and one produced triplets. The production of more than one hatchling from an egg has also been seen in other species of *Ariolimax* (Leonard *et al.*, unpubl. data; B. Miller, pers. comm.). Clutch 1C produced 18 hatchlings 24-28 March 2005.

The second individual (from a clutch found 28 November 2003) laid a total of 120 eggs, 101 which hatched: a) 28 eggs found 12 October 2005, 16 of which hatched; b) 17 eggs found 18 November 2005, 18 of which hatched, including one set of twins; c) 56 eggs found 8 December 2005, 50 of which hatched; and d) 19 eggs found 17 February 2006, 17 of which hatched. The third individual, from a clutch of 13 November 2003 [a clutch mate of the first individual (above)], was found with a clutch of 80 eggs on 8 December 2005, which produced 66 hatchlings, and a clutch of 56 eggs on 19 January 2006 which produced 55 hatchlings. The latter two individuals survived until frozen on 22 June 2006, and were found to be aphallate when dissected (Table 2).

A box of four individuals, two from Staten Island parents and two from Tomales Bay parents produced a clutch of 17 eggs on 14 October 2004. The oldest individual in the box

Table 2. Summary of dissections of *Ariolimax buttoni* held at UCSC at Santa Cruz, California

Population	Isolate/Group-held/Collected?	Penis	Vas deferens	Spermatheca	Egg laying
Central Marin	Collected as adult	Very large bulbous	Connects to penis		Never laid eggs in lab
UCSF	Collected as adult	Very thick	Connects to penis	Sac-like	Never laid eggs in lab
Staten Island	Collected as adult	No trace of penis	Blind termination	Reddish, flaccid	Never laid eggs in lab
Staten Island	Reared in isolation throughout life	Small penial stub	Blind termination	Reddish	Laid fertile eggs
Staten Island	Reared in isolation throughout life	Small penial stub	Blind termination	Reddish, sac-like	Laid fertile eggs
Staten Island	Laboratory reared, paired at 6 months of age	Splayed penial stub	Blind termination	Dark red	3 clutches of fertile eggs from pair
Staten Island	Laboratory reared, paired at 6 months of age	No trace of penis	Ends in genital pore		3 clutches of fertile eggs from pair
Staten Island	Laboratory reared, paired at 6 months of age	No trace of penis	Connects to base of penial stub near atrium	Red, flaccid	Fertile eggs laid by pair
Staten Island	Laboratory reared, grouped at 6 months of age	No trace of penis; 1 cm of vagina everted	Blind termination	Large	Several clutches of fertile eggs laid by group
Staten Island	Laboratory reared, grouped at 6 months of age	Small penial bulb	Blind termination	Dark reddish	Several clutches of fertile eggs laid by group
Staten Island	Laboratory reared, grouped at 6 months of age	No trace of penis	Ends in bulb		Several clutches of fertile eggs laid by group
Staten Island	Laboratory reared, grouped at 6 months of age	No trace of penis; 0.5 cm of genital pore everted	Blind termination	Large, flaccid, red	Several clutches of fertile eggs laid by group
Tomales Bay	Reared in isolation throughout life	Penis present	Connects to penis	Flabby, small	Never laid eggs
Tomales Bay	Reared in isolation throughout life	Penis present	Connects to penis	Reddish, moderately small	Never laid eggs
Tomales Bay	Reared in isolation throughout life	Penis present	Connects to penis	Small, pale	Never laid eggs
Tomales Bay	Laboratory reared, paired at 6 months of age	Penis present	Connects to penis	Small, creamy, firm	Never laid eggs

came from a clutch of 13 November 2004, so the parent was no more than 11 months old at the time of egg-laying. This clutch produced 3 hatchlings. On 11 November 2004, a clutch of 29 eggs was found in a box containing a pair of individuals, both from Tomales Bay parents, one from a clutch found 30 December 2003 and the other from a clutch found 2 January 2004. This means that the individual laying the eggs could not have been older than 10.5 months from the date the egg was laid. Fourteen of the 29 eggs in this clutch hatched. A second clutch of 12 eggs found in this box on 13 December 2004 produced 6 hatchlings. On 24 November 2004 the Staten Island (spotted) individual of a pair of one Staten Island (spotted) and one Tomales Bay (im-

maculate) slug, was found laying eggs. A total of 54 eggs were found in the box at that time, although it is not clear that they were all laid by the spotted mother. Forty-one hatchlings were produced from this clutch. The spotted slug was hatched from a clutch found 16 January 2004 and so was approx. 10 months and eight days in age (from the egg). Nine more eggs were found in this box on 24 November 2004 and this clutch produced 2 hatchlings.

Sexual Behavior

Little is known about sexual behavior in *Ariolimax buttoni*. To date, we have never observed a complete sexual interaction from courtship to the termination of copulation
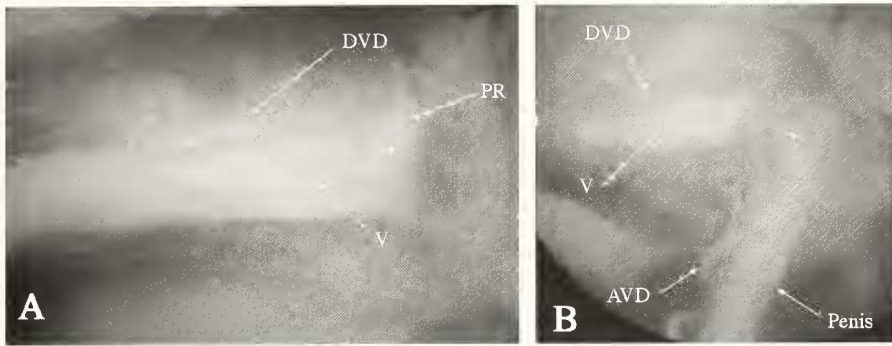
Figure 2. Photographs of dissections of aphallate (A) and euphallate (B) individuals of *Ariolimax buttoni*. Individual A was laboratory reared from Staten Island parents. Individual B was laboratory reared from Tomales Bay parents. AVD, ascending vas deferens; DVD, descending vas deferens; P, penis; PR, penis stub; V, vagina; *, location of atrium.
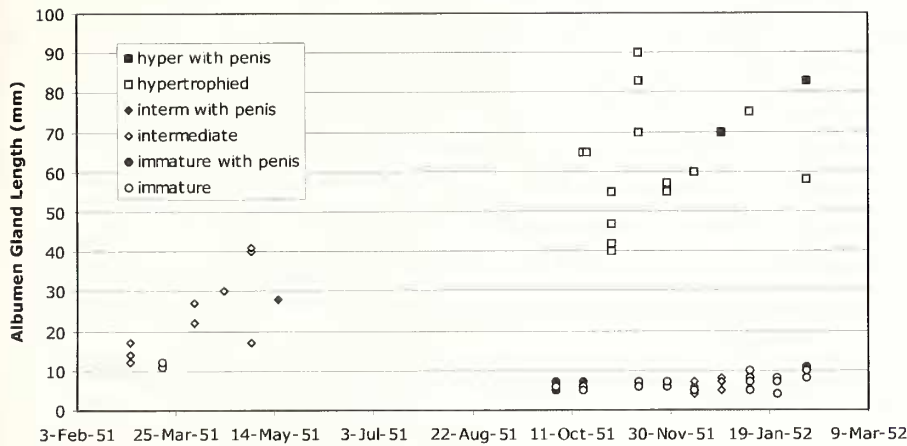

Figure 3. Relationship between reproductive state (Appendix), season, and albumen gland length in 66 individuals *Ariolimax buttoni.*

in this species. However, UCSC animals maintained in the laboratory in groups have occasionally been found *in copula*. *Ariolimax buttoni*, like *A. columbianus,* and unlike other species of *Ariolimax*, has both spotted (maculate) and unspotted (immaculate) individuals, and three of the copulating pairs observed in this study involved both a maculate and an immaculate individual (Fig. 5; Table 3). Copulations between maculate and immaculate individuals are also seen in *A. columbianus* (Cody 2006).

In the laboratory, copulations have been observed from February through early September. Copulation was observed on 11 or 12 (see below) occasions involving animals from 5 different populations (Table 3). In one case, a pair of slugs from Central Marin was found copulating unilaterally at 11:07 pm on 5 September 2002 (by JSP), observed until 12:30 am 6 September 2002, and then left alone overnight. What was apparently the same pair of slugs, in the same position, was found copulating when the box was next checked at 2:30 pm on 6 September 2002 and the pair finally separated at 10 pm that evening. We cannot say with confidence whether this represents one or two copulations. If we count it as two copulations, of the 12 copulations that have been observed, 7 involved simultaneously reciprocal copulation between the members of a pair whereas 5 were uni-

lateral. If the interactions of 5-6 September 2002 are counted as one copulation, then 7/11 copulations observed have been simultaneously reciprocal. In three cases, the termination of the copulation was observed and in all of these cases, the copulation was unilateral when first observed. Of these, one terminated 62 min after the copulation was noticed, one terminated 98 min after first observed and one (5-6 September 2002) terminated 7.5 h after observation resumed on the second day; 22 h and 53 min after the pair was first seen copulating. Reciprocal copulations may become unilateral when one penis is withdrawn considerably earlier than the other (Leonard *et al.* 2002). The available data (Table 3) show that copulations in *Ariolimax buttoni* are often very long. In 3/12 observations copulation lasted more than 7 h, and in one case (5-6 September 2002), the interaction may have lasted almost 23 h. In the unilateral copulation on 5 February 2001, between two immaculate individuals collected from the UCSF campus, when the pair were first seen, one slug had intromission as a male and the second slug (acting as female) had its penis completely everted and resting against the body of its partner. The end of the penis was greatly enlarged in the form of a broad bulb. As the second slug withdrew its penis, the bulb gradually deflated and the penis involuted from the tip. In the observations of 5-6
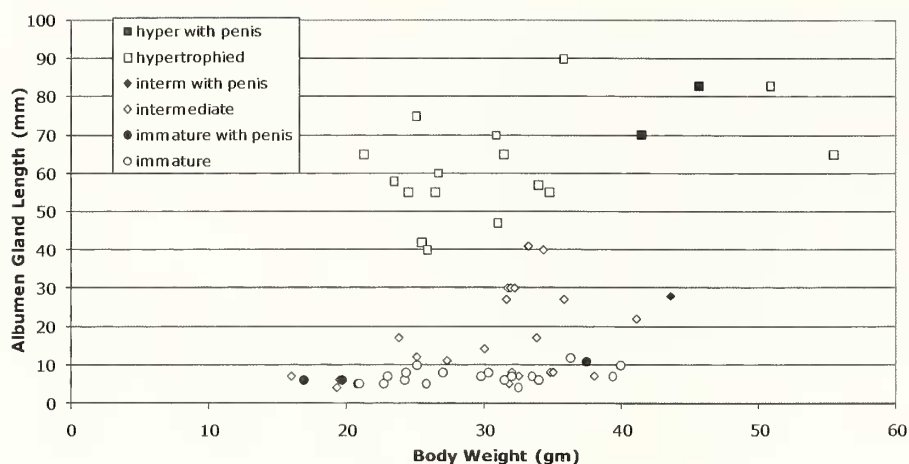
Figure 4. Relationship between reproductive state (Appendix), body weight, and albumen gland length in 66 individuals of *Ariolimax buttoni*.

Table 3. Copulations of *Ariolimax buttoni* from different populations in California.

Population	Date	Time first observed	Observations ended	Type of intromission
UCSF	2/5/2001	16:05	17:43	Unilateral
Mendocino	4/10/2001	17:45	17:46	Simultaneously reciprocal
Mendocino	5/3/2001	17:30	20:29	Simultaneously reciprocal
Mendocino	5/17/2001	21:05	22:23	Simultaneously reciprocal
Central Marin	7/17/2002	13:15	15:17	Simultaneously reciprocal
Central Marin	9/5/2002	23:07	24:30	Unilateral, spotted male
Central Marin	9/6/2002	14:30	22:00	Unilateral, spotted male
UCSF	4/8/2003	18:31	26:01	Simultaneously reciprocal
UCSF	5/5/2003	11:52	23:35	Simultaneously reciprocal
Tomales Bay	5/6/2003	16:04	18:28	Unilateral
Tomales Bay	6/19/2003	15:45	16:47	Unilateral
Tomales Bay	8/8/2003	13:44	18:30	Simultaneously reciprocal

September 2002 one slug was seen to contact the penis with its mouth on several occasions but there was no evidence of chewing on the penis.

DISCUSSION

Phally Polymorphism

Since Heath's (1916) description of apophallation in *Ariolimax californicus*, the tendency has been to explain absence of a penis in this genus in this way (Mead 1942, 1943). However, Heath himself expressed doubt of this interpretation (cited in Mead 1943, see above). The current study was stimulated by the observation that all 27 individuals of *Ariolimax* collected on the Mills College campus for an anatomical study lacked a penis (Paull 1951). This is a higher incidence of aphally than would be expected from the 5% apophally rate reported by Heath (1916) for *A. californicus*. Only 7 of 67 slugs collected from Mills College in the current

study had a penis. In all cases the aphallate slugs were lacking not only a penis but all penial musculature, and the vas deferens was connected to tissue at or near the atrium (Fig. 1), terminating in a bulb in many cases, which is not what one would expect from apophallation (see discussion by Roth 2004). Further evidence that aphally in this population is not derived from apophallation during copulation comes from the observation that none of a series of sexually immature laboratory-reared slugs from Mills College, which were up to 4 months old, showed any signs of development of a penis. This led to the hypothesis that aphally occurred naturally in some individuals of *A. buttoni*, and that an innate phally polymorphism rather than apophallation was largely responsible for the presence or absence of a penis in this and perhaps other species of *Ariolimax*.

This hypothesis was confirmed by the observation that in individuals derived from the Staten Island population, aphally was found even in two individuals that were reared in isolation from the egg to the age of 30 months and had

both laid eggs. Aphally in these individuals could not be due to either apophallation or sexual immaturity. Moreover, the anatomy of these aphallic isolates was consistent both with that of other Staten Island individuals that were group-housed as adults and with that of the Mills College animals. The aphallates are characterized by either the complete absence of a penis (Fig. 1) or reduction of the penis to a small stub (Fig. 2A); in both cases, the penial retractor muscle is absent and the vas deferens ends blindly in a mesentery or in the neighborhood of the atrium. The description of dissection of a freshly apophallate *A. dolichophallus* by Mead (1942) suggests, however, that the distinctions between aphally as described in Fig. 1 and 2, and a healed apophallate individual, are subtle.

The anatomy of *Ariolimax buttoni*, as described by Pilsbry and Vanatta (1896), and the aphallate individuals from Mills College described here (Fig. 1, also Paull 1951) and the Staten Island animals (Fig. 2A), is very consistent with the descriptions and illustrations of aphallic individuals in other stylommatophorans (Watson 1934, Riedel 1955, Tompa 1984, Pokryszko 1987, see discussion in Roth 2004). Phally polymorphism, in which a hermaphroditic population or species consists of a mixture of individuals with normal penes (euphallic individuals) and individuals with either markedly reduced penes (hemiphallic individuals) or no penis at all (aphallic individuals) is widespread in the Pulmonata and has evolved many times (see discussion in Duncan 1975, Tompa 1984, Pokryszko 1990, Lace 1992, Schrag and Read 1992, Viard *et al.* 1997, Doums *et al.* 1998, Backeljau *et al.* 2001). The results presented in this study offer a clear demonstration of phally polymorphism in *A. buttoni*.

Aphally seems to be common in at least some populations of *Ariolimax buttoni*. Pilsbry and Vanatta (1896, discussion in Pilsbry 1948) examined a large collection of individuals lacking a penis from Oakland, and Heath (Mead 1942, cited above) dissected 400 aphallate specimens from Hog Island in Tomales Bay. Westfall (1952) also reported that of 85 specimens collected at Mills College in the spring of 1952 (subsequent to the work described here), only 9 had a penis, of which 3 were in the hypertrophied state and 6 in the immature reproductive state, in the terminology used here. It is characteristic of phally polymorphisms that the percentage of a given morph varies widely from one area to another and from season to season (Lace 1992). Baur and Chen (1993) found that the frequency of aphallic individuals in populations of *Chrondrina avenacea* (Bruguière) varied from 0.9% to 89.2% in the vicinity of Basel, Switzerland. The tendency for the frequency of phally morphs to vary with environmental conditions (*e.g.*, Watson 1934, Schrag and Read 1992, Baur *et al.* 1993) may explain why Mead (1942, 1943) found only euphallate individuals in the vicinity of Hog Island whereas Heath had found only aphallate individuals at Hog Island earlier. In this study, all four individuals derived from populations near Tomales Bay were euphallate (Table 2).

The results presented here, then, demonstrate that phally polymorphism is present in *Ariolimax buttoni*, showing that only 7/67 Mills College and 4/16 UCSC, including 0/10 Staten Island, slugs had a penis, and leave open the question of whether apophallation occurs in this species. Aphallate individuals have also been found in a population of *Ariolimax (Meadarion) brachyphallus* from Hillsborough, San Mateo City, CA (Pearse, *unpubl. data*). Wright (1938) stated that 415 individuals of *Ariolimax californicus* he examined did not have a penis, or other "male parts" whereas 248 individuals were found to have normal, "or regenerating" penes. Wright also stated that the aphallic individuals were the result of apophallation but provided no evidence to support this, nor did he provide details of the anatomy or even information as to the source of these specimens. Wright's observation suggests that an anatomical study of that species is needed to determine whether aphally as well as apophallation occurs. It is possible that phally polymorphism will be found to be more widespread in *Ariolimax*.

The adaptive significance of phally polymorphism is not entirely clear. Local Mate Competition theory (Charnov 1982) predicts that allocation to male function in hermaphrodites should be reduced where few sexual partners are available, suggesting that aphally should be more common where self-fertilization is common or population densities are low. Baur *et al.* (1993) hypothesized that aphally evolves in populations that typically self-fertilize. In both cases, aphally is predicted to be associated with a capacity for uniparental reproduction and the role of environmental factors in influencing the ratio of aphallic to euphallic offspring is hypothesized to be an adaptation to colonizing new habitats (Schrag and Read 1992, but see Baur *et al.* 1993)

Uniparental Reproduction

Uniparental reproduction, either by self-fertilization, as has been widely assumed, and well-documented in some cases, or by apomixis, as suggested by some authors (McCracken and Selander 1980, Foltz *et al.* 1982a, Hoffmann 1983) is widespread, although not universal in stylommatophorans (Tompa 1984, Heller 2001). Within a genus, some species may be obligate outcrossers whereas others readily self-fertilize (Foltz *et al.* 1982b, Reise 2002). Mead (1942) reared *Ariolimax* in isolation for as long as two years without the production of any eggs but considered the question of uniparental reproduction in *Ariolimax* still open. Here we report that *A. buttoni* can produce viable offspring with high rates of hatching success without cross-fertilization. Uniparental reproduction has also been observed in *A. dolichophallus* but with low hatching success (Miller and Sinervo 2007). Westfall (1952) reported that in Mills College animals, the development of the ovotestis did not differ be-

tween euphallic and aphallic individuals; that is the qualitative degree of sperm vs. egg production did not appear to depend on phally status. The production of sperm observed in aphallate individuals in histological studies (Westfall, 1952), suggests that self-fertilization could occur in *A. buttoni*. Similar results have been reported in the genus *Zonitoides* Lehman, 1862 (Watson 1934). Resolution of the question of whether these offspring are the result of selfing or apomixis will require genetic analysis.

Sexual Behavior

Of the observations reported in Table 3, about half of the pairs had reciprocal intromissions and the other half had unilateral intromissions, in which one slug was acting as a female and the other as a male. Copulation lasted more than 7 h after being first observed in 3 of 12 observations, and in one case (5-6 September 2002) the interaction may have lasted almost 23 h after detection (see above; Table 3). Thus, copulation in *Ariolimax buttoni* often lasts more than the two hours typical of simultaneously reciprocal intromissions of *A. dolichophallus* and much longer than the brief, unilateral intromissions of *A. californicus* (Leonard *et al.* 2002). In long reciprocal copulations such as those of *A. dolichophallus* (Leonard *et al.* 2002) it is not unusual for one individual to withdraw the penis long before the other (Leonard, unpublished observation). Therefore, it seems likely that copulations between euphallate *A. buttoni* (Figs. 5A-C) are normally long and simultaneously reciprocal. The unilateral intromissions observed here may have been the end of simultaneously reciprocal copulations or they may have involved copulation with a partner that lacked a penis (below).

Life History

The life histories of *Ariolimax* spp. are poorly known. The observations reported here provide the most detailed picture available on sexual development and the reproductive cycle for any species of *Ariolimax*. We found that, in the laboratory, *Ariolimax buttoni* may live more than 30 months. This is consistent with previous reports that individuals of *A. californicus* and *A. dolichophallus*, collected as adults, survived more than 18 months in the laboratory (Leonard *et al.* 2002). The anatomical and histological data obtained in the Mills College study indicate that, in Oakland populations, individuals may live more than a year in the field, since two classes of individual were found in fall and winter dissections; spring and summer specimens showed a gradual increase in albumen gland length, suggesting the maturation of a single cohort (Fig. 3). This fits well with field data (Pearson *et al.* 2006) for identifiable individuals in a population of *A. buttoni* in Orinda, Contra Costa Cty, CA that showed a life span of approx. 2 years.

The data also provide a clear picture of the phenology of *Ariolimax buttoni*. Both UCSC (Leonard *et al.* unpublished)

and Mills College animals held in the laboratory (Westfall 1952, 1960) laid eggs in the fall and winter as suggested for *Ariolimax* by Mead (1942). Mills College animals with reddish-brown ovotestes, a spermatheca filled with reddish fluid, and a yellow albumen gland larger than the ovotestis (the hypertrophied reproductive state), believed to represent the egg-laying stage, were first seen in a dissection of 22 October 1951 and then seen regularly until February 1952 (Fig. 3). Egg-laying and juvenile growth from individuals in the Mills College portion of the study have been reported elsewhere (Westfall 1960). Westfall (1960) reported a minimum time to hatching of 23 days and a maximum of 2 months for Mills College slugs, which brackets the 47-54 days found for UCSC animals (Leonard *et al.* unpublished). In *A. dolichophallus*, hatching time ranged from 51-55 days and in *A. californicus* from 46-81 days (Leonard *et al.* 2002). These hatching times all reflect laboratory conditions and will probably depend strongly on temperature, making it likely that hatching times in the field will be somewhat longer. In the spring and summer, animals were found to be in the immature and intermediate reproductive states, whereas in fall and winter all three states were found (Fig. 3). This is consistent with data from Paull (1951) who noticed that the reproductive system was more often in the immature state from February to April, whereas hypertrophy of the reproductive tract was predominant in *A. buttoni* collected from September to the middle of February. In field observations, juveniles of *A. buttoni* weighing less than 1 gram appeared in January (Pearson *et al.* 2006).

Since laboratory data demonstrate that *Ariolimax buttoni* can live more than one year, with some individuals laying eggs at less than one year of age while some clutch mates reared under the same conditions do not lay eggs until over two years of age (see below), it seems likely that not all individuals will reproduce in their first year, perhaps accounting for the occurrence of individuals in the immature and intermediate reproductive states throughout the year in this study. Protandrous development of the ovotestis is typical of stylommatophorans (Tompa 1984) and has been reported for *A. californicus* (Gottfried and Dorfman 1970), making it seem probable that development in *A. buttoni* may involve a progression from the immature reproductive state through the intermediate male state to the hypertrophied female state, and then the old reproductive state, although the intermediate state may be heterogeneous. The intermediate reproductive state is the state characterized by massive sperm production (Westfall 1952; Appendix) and one would expect it to be associated with copulation. Our observations suggest that in *A. buttoni* may become sexually mature and begin copulation as early as the summer of their first year and lay eggs that same fall. The first specimen found to have an albumen gland greater than 20 mm in length in the Mills College study was dissected in April 1951 (Fig. 3). The first

Figure 5. A. A copulating pair of euphallate individuals of *Ariolimax buttoni*. Both individuals were collected from the UCSF campus and held in a group box; found copulating when the box was opened for cleaning. B. The same pair of individuals later in same copulation, with at least one penis inflated. C. One individual of same copulation with short, rigid withdrawn penis still everted after withdrawal from partner.

individual found to have a reddish and enlarged spermatheca (= bursa copulatrix = gametolytic gland) was dissected 17 October 1951. The spermatheca, often termed the gametolytic gland (Tompa 1984, Gómez 2001), serves to digest both allo- and autosperm in stylommatophorans, as well as stray oocytes and excess secretions. The reddish color of the spermatheca may, therefore, indicate copulation and/ or egg-laying. Individuals from the Staten Island population that were reared in isolation but had laid eggs had a reddish spermatheca (Table 2) but one of the individuals from To-

males Bay, also reared in isolation but having never laid eggs, also had a reddish spermatheca. Laboratory observations from UCSC animals (Table 3) show copulations occurring between February and September. This is consistent with field observations from *A. dolichophallus* in which observations of copulating pairs ranged from February to mid-October (Leonard *et al.* 2002).

Laboratory data from the UCSC animals indicate that the rate of maturation, at least in terms of egg-laying, varies widely. The age at first egg-laying has varied from slightly

over 10 months to more than 24 months among individuals that have laid at least one clutch and many animals in the study had not yet laid eggs at 30 months of age (Leonard *et al.* unpublished), as Mead (1942) also reported. Gottfried and Dorfman (1970), reported that individuals from a population of *Ariolimax californicus* from Portola Valley, San Mateo Co., California, reared in the laboratory, showed protandrous gonadal maturation with individuals having immature gonads at body weights of less than 10 g and individuals of 20-30 g and approx. 12 months of age having "maturing male phase" gonads. The first "intersex" gonads appeared at a body weight of 40-45 g and an approx. age of 24 months and fully female gonads were associated with a body weight of 55-60 g in weight and 36 months of age. These data are similar to those from individuals in the current study (Staten Island isolates, Table 2) that first laid eggs at two years of age, although there was in general no clear relationship between weight and reproductive state in the Mills College animals (Table 1; Figure 4). In a study of identifiable individuals in the field, Pearson *et al.* (2006) found a correlation between age and weight only up to 20 g for *A. buttoni*. Gottfried and Dorfman's (1970) data differ substantially from the results obtained for *A. californicus* by Leonard *et al.* (2002) using the same rearing conditions as used for the UCSC animals in the current study, where lab-hatched individuals were found to begin copulating at 8 months of age, laying eggs as early as 50 weeks of age, and achieving a much higher growth rate than reported by Gottfried and Dorfman (1970). Lab-reared *A. californicus* were observed to copulate as early as eight months of age (from the egg) and to lay eggs at 12 months (Leonard *et al.* 2002) and *A. (Ariolimax) stramineus* Hemphill, 1891 and *A. (Meadarion) brachyphallus* Mead, 1943 have also been observed to lay eggs at less than 12 mo of age in the laboratory (Leonard *et al.* unpublished). In contrast, Mead (1942, p. 116) reported that the genital system was still "very minute and completely non-functional" in *A. brachyphallus* at the end of one year and that sexual maturation (ability to copulate as a male and to receive sperm but not lay eggs) was reached in *A. dolichophallus* at approx. 18 mo. Miller and Sinervo (2007) reported large variance in growth of *A. dolichophallus* in the laboratory, as seen by Leonard *et al.* (2002) for *A. californicus*. This may be associated with variance in sexual development as seen here in *A. buttoni*.

Reproduction in stylommatophorans appears to be strongly influenced by environmental factors (Potts 1975, Tompa 1984, South 1992, Gomot de Vaufleury 2001). Moreover, growth rates may vary greatly among clutch mates reared under the same conditions (Shibata and Rollo 1988, Leonard *et al.* 2002, Miller and Sinervo 2007), and, as seen in the current study, the age at first egg-laying may vary by more than 12 months among clutch mates. Consequently,

variation from year to year and site to site would not be unexpected in *Ariolimax buttoni*. However, in general the life cycle seems to involve egg-laying in fall and winter with hatching in late winter to early spring, and some individuals copulating as early as the late summer or fall of their first year and laying eggs in the late fall or winter. Overwintering individuals may copulate in the spring or summer of the second year and lay eggs that fall or winter. Whether regression of gonads occurs in *A. buttoni* (see discussion in Mead 1942, Westfall 1952) is not clear. Also, we do not know whether an individual will lay eggs in successive years. Some stylommatophorans have an iteroparous life cycle, with cycles of gonadal development and reproduction in successive years whereas other species have a pattern whereby individuals have a more or less prolonged semelparous life cycle (perhaps over several years) and gonadal development is not reversible once sexually mature (see discussion in Heller 2001). The available data do not allow us to distinguish between these models for *A. buttoni*, or other species of *Ariolimax*.

Sexual Selection and the Sexual Biology of *Ariolimax buttoni*

In order to understand the potential for sexual selection, it is important to understand the age at first reproduction, the duration of the reproductive life span, the type of mating behavior, and the potential for uniparental reproduction. The potential strength of sexual selection is measured by the variance in reproductive success among individuals (see review in Leonard 2006), and one factor that would tend to increase the potential for sexual selection in a species is a skewed sex ratio. While in simultaneous hermaphrodites the operational sex ratio, or sex ratio at the time of mating, is considered to be 1:1, if there were a delay between mating and egg-laying there may be a skew in breeding sex ratio (Arnold and Duvall 1994). In *Ariolimax*, *A. californicus* have been observed to copulate as young as 8 months of age and to begin egg laying at 11.5 months of age (Leonard *et al.* 2002). This delay suggests that not all individuals that copulate will lay eggs, creating a potential skew between the number of individuals in the population that sire young and those that are mothers of young, which would tend to increase the variance in reproductive success among individuals and the potential for sexual selection. The range in age at first egg-laying seen in *A. buttoni* in the current study (from 10 to more than 24 months) further suggests that variance in female reproductive success will be substantial. Phally polymorphism may also contribute to skewed breeding or even operational sex ratios in that aphallates will be unable to copulate as males with other individuals although they may be able to copulate as females (but see Reise 2007). The anatomy of aphallic individuals in *A. buttoni*, with the vas

deferens ending blindly or in a bulb seems to be inconsistent with transfer of sperm to conspecifics. However, observations of copulation in the female role by apophallate *A. dolichophallus* (Leonard *et al.* 2002) suggest that aphallate individuals may be able to copulate as females, receiving sperm from euphallate partners. In the stylommatophoran, *Vertigo pusilla* O.F. Müller, 1774, Pokryzsko (1990) found that aphallates copulated as females with euphallate individuals. In the basommatophoran *Bulinus truncatus*, in which self-fertilization is the predominant mode of reproduction, phally status (euphallic or aphallic) was not correlated with offspring heterozygosity, indicating that aphallic individuals are as likely to have their eggs fertilized by a conspecific as were euphallic individuals (Doums *et al.* 1996, Viard *et al.* 1997). In a mixed population of aphallates and euphallates with outcrossing, the euphallates should have greater potential reproductive success in the male role, increasing variance in reproductive success and the potential for sexual selection. However, if reproduction in *A. buttoni* is predominantly uniparental, the opportunity for sexual selection would be limited.

Sexual behavior also offers indications of the strength of sexual selection. The very long (>7 h) copulations observed in this study (Table 3) suggest that sexual behavior in *Ariolimax buttoni* may involve higher expense and/or risk than in species with shorter copulations. Sexual selection in hermaphrodites has been hypothesized to involve resolution through reciprocity between members of a pair of a sexual conflict over sexual role (Axelrod and Hamilton 1981, Leonard 1990, recent reviews by Michiels 1998, Leonard 2005, 2006). In *A. dolichophallus* the mating system is based on simultaneously reciprocal copulation between the members of a pair (Leonard *et al.* 2002). In *A. californicus* intromissions are unilateral but occur in bouts and the members of a pair are hypothesized to alternate roles during these bouts, creating reciprocity (Leonard *et al.* 2002). In the data presented here, about half of the observed copulations were simultaneously reciprocal (Fig. 5A) and since in other species of *Ariolimax* simultaneously reciprocal copulations often involve a period of unilateral intromission after one individual withdraws (Leonard *et al.* 2002, Leonard and Pearse, *unpubl. obs.*), we hypothesize that simultaneously reciprocal copulations will be found to be the rule in *A. buttoni* where both individuals are euphallate.

If reciprocity were important to the mating system, as predicted by the Hermaphrodite's Dilemma model (Leonard 1990), then individuals able to mate in only one sexual role, as is assumed to be the case for aphallates (but see Westfall 1952), should be less desirable as mating partners than euphallates. If on the other hand, the reciprocity seen between euphallates is "unconditional" and an artifact of both hermaphrodites being eager to act as males as predicted by

Michiels (1998, see discussion in Leonard 2005), then aphallates should be as desirable as euphallates as sexual partners. *Ariolimax buttoni*, offers an exciting opportunity to distinguish between these models. Additionally, the capacity for uniparental reproduction should allow hermaphrodites to avoid Game of Chicken conditions in the Hermaphrodite's Dilemma (Leonard 1990). Therefore, in a species with the option of uniparental reproduction, the Hermaphrodite's Dilemma predicts that reciprocity will be conditional on the partner's behavior (see Leonard 2005). The evidence for phally polymorphism and uniparental reproduction in *A. buttoni* creates an interesting opportunity to test the predictions of theory. Genetic studies will be required to distinguish between hypotheses and to measure the variance in reproductive success and hence, potential for sexual selection in *Ariolimax*.

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APPENDIX

Definitions of Reproductive States in *Ariolimax buttoni*
(Histological data from Westfall 1952)

1. **Immature State**: Reproductive system located anteriorly, close to atrium; ovotestis (=hermaphroditic gland = gonad), white, often transparent; small, thin hermaphroditic duct; spermatheca (=bursa copulatrix = gametolytic gland) flattened, transparent; albumen gland < 20 mm in length, usually smaller than the ovotestes, made up of small, white lobes, close to and alongside ovotestis. Histologically, spermatozoa absent; abundant spermatogonia and spermatocytes, only occasional groups of spermatids; germinal epithelium well-formed, lumen without much connective tissue; fair number of eggs in various stages of development and degeneration. The reproductive system in the immature state resembles that of juvenile slugs.

2. **Intermediate State.** Ovotestis smooth, creamy, solid; greatly expanded hermaphroditic duct; spermatheca, colorless; albumen gland white or sometimes yellow, appearance as immature state. Histologically, cross section with sperm predominant in ovotestes, characteristically arranged in orderly fashion around inner periphery of each acinus; sperm massed in hermaphroditic duct; a few eggs in various stages of development; large oocytes protrude from inner walls of the acini; oocytes easily distinguished by large amount of cytosome in proportion to size of distinct, round, clear nucleus; nucleolus of oocyte usually stains darkly; degenerating oocytes fairly common, appear yolky, usually irregular in outline.

3. **Hypertrophied State.** Ovotestis dark, reddish brown with granular appearance; hermaphroditic duct, convoluted and yellow, pushing ovotestis and albumen glands posteriorly, filled with masses of spermatozoa; spermatheca bulges with red fluid; albumen gland very large (> 20 mm), larger than ovotestis, extending posteriorly, filling up much of the body cavity. The one slug taken for dissection while egg-laying was in the hypertrophied state with oviduct distended with eggs and masses of sperm in hermaphroditic duct. Histologically, ovotestis cross section somewhat ragged, acini noticeably shrunken and detached, separated by wide spaces within ovotestis membranes; all stages of spermatogenesis present, although spermatozoa fewer than in intermediate stage and no longer arrayed in strikingly regular fashion of the intermediate state; eggs large, relatively rare; most ova degenerating, apparently left over from spawning. Portion of hermaphroditic duct visible in ovotestis cross sections shrunken, sperm masses visible only in smears under the microscope.

4. **"Old" State.** "Six dehydrated and wrinkled specimens from a moist terrarium which were dissected had atrophied reproductive organs with straight ducts, but in all cases the hermaphroditic gland was a light reddish-brown color. In three of these specimens the spermatheca seemed white and cloudy. Smears on microscope slides showed that the spermathecae were filled with spermatozoa" (Westfall 1952, p. 46).